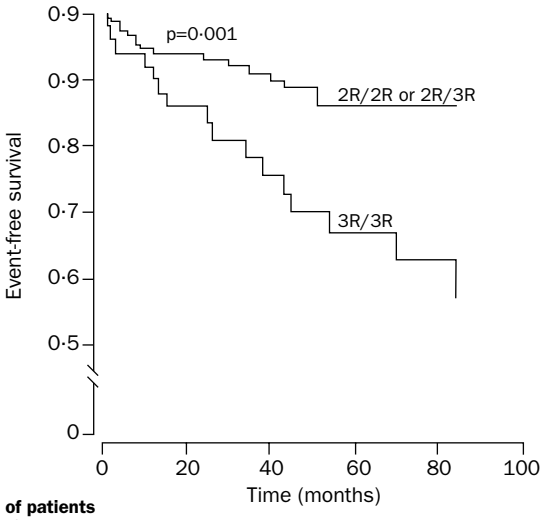

Number of patients remaining						
3R/3R	50	35	26	16	12	0
2R/2R or 2R/3R	155	114	80	51	31	0

Kaplan-Meier estimates of event-free survival for patients with and without thymidylate synthase 3R/3R genotype

dependent variable (p=0·002) or when patients who received low-dose methotrexate in the DFCI 87-01 protocol (n=22) were added to the analysis (p=0·003). Hazard ratio was estimated with Cox proportional hazard model in the presence of clinical prognostic factors (hazard ratio=3·3, 95% CI 1·4–7·8, p=0·006) Further to thymidylate synthase genotype, which was a major contributor to risk of event, we also saw an association of WBC count greater than 50×10^9/L with worse outcome of acute lymphoblastic leukaemia (p=0·02). No effect of genotype on any clinical prognostic factors was noticed (data not shown).

Acute lymphoblastic leukaemia is the most frequent malignancy that affects children. Resistant cases and long-term effects caused by intensive chemotherapy suggest a need for alternative treatments. The key drug in the treatment of this leukaemia is methotrexate, which—by synthesis of methotrexate glutamates—acts as an inhibitor of thymidylate synthase.[2] Interindividual diversity in response to methotrexate, along with variability in thymidylate synthase mRNA expression, is documented.[2] This variation could interfere with drug response and treatment efficacy.

We report that homozygosity for the thymidylate synthase triple repeat, which is known to correlate with raised thymidylate synthase expression,[4] is associated with poorer outlook in children with acute lymphoblastic leukaemia than in those with at least one double-repeat allele. A closely similar finding has been reported for patients with colon cancer who received chemotherapy.[5] Overexpression of thymidylate synthase might be a mechanism by which individuals with the 3R/3R genotype develop resistance. This finding is in accordance with observations that raised concentrations of thymidylate synthase predict a poor response to inhibitors of thymidylate synthase in various carcinomas.[1] Furthermore, in myeloid or T lymphoblastic leukaemic cells that are less sensitive to methotrexate than B cells, increased expression of thymidylate synthase was reported.[2]

In conclusion, thymidylate synthase genotype seems to be a significant predictor of outcome in childhood acute lymphoblastic leukaemia. This finding could lead to individualisation of treatment for patients with this disease.

Contributors

I Costea and S Chiasson were responsible for genotyping of thymidylate synthase polymorphisms and retrieval of clinical data. Maja Krajinovic was responsible for study design, data analysis, and the writing of the report.

Conflict of interest statement

None declared.

Acknowledgments

We thank Daniel Sinnett and Damian Labuda for biological material and discussion; and Albert Moghrabi and Mark Bernstein for access to clinical data. The equipment and reagents were purchased by funds provided by the Leukemia Research Fund of Canada and Centre de Recherche, Hôpital Ste-Justine. The funding sources had no role in study design, data collection, data analysis, data interpretation, or writing of the report. MK is a scholar of the Fonds de la Recherche en Santé du Québec.

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Antibodies to varicella-zoster virus in blood donors with genetic variance in CC chemokine receptor 5

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Carriers of a 32 bp deletion (*Δ32*) allele of the CC chemokine receptor 5 (*CCR5*) gene are reported to be more likely to lack antibodies to varicella-zoster virus than *CCR5* wild-type individuals. To find out whether *CCR5-Δ32* is associated with the seroprevalence of varicella-zoster virus infection, we tested blood donors with different *CCR5-Δ32* genotypes for varicella-zoster virus IgG. Antibody to varicella-zoster virus was present in 209 (99·5%) of 210 *CCR5-Δ32* carriers and exactly the same proportion of *CCR5* wild-type individuals (209 of 210). We have therefore found no evidence that the *CCR5-Δ32* allele is associated with decreased seroprevalence of varicella-zoster virus infection.

Lancet 2002: **359:** 1034–36

Chemokines and chemokine receptors play an important part in the inflammatory response by mediating chemotaxis and leucocyte activation. CC chemokine receptor 5 (CCR5) also serves as the major coreceptor for HIV-1 strains that predominate in the early phase of infection.[1] An allele with a 32 bp deletion (*Δ32*) in the *CCR5* gene confers resistance

	CCR5-Δ32 homozygotes (n=14)	*CCR5-Δ32* heterozygotes (n=196)	Wild-type controls (n=210)
Number positive for VZV IgG in serum	13 (92·9%)	196 (100·0%)	209 (99·5%)
Optical density on ELISA			
Low	0	2 (1·0%)	2 (1·0%)
Moderate	0	6 (3·1%)	5 (2·4%)
High	13 (100%)	188 (95·9%)	202 (96·7%)

IgG against varicella-zoster virus (VZV) in serum of blood donors by *CCR5-Δ32* genotype

to HIV-1 infection and slows disease progression.[2] Observations regarding the origin of the *CCR5-Δ32* allele suggest that it might offer protection against at least one other infectious disease, which has not yet been determined.[3]

Because chemokines and chemokine receptors are essential for defence against infectious pathogens, mutations in chemokine receptor genes might modulate the host's response to other virus infections. Wiencke and colleagues reported that *CCR5-Δ32* carriers (heterozygotes and homozygotes) were 9·2 times more likely to be seronegative for antibody to varicella-zoster virus than were *CCR5* wild-type individuals. That study of 157 normal adult blood donors included two *CCR5-Δ32* homozygotes and 27 *CCR5-Δ32* heterozygotes.[4] We did a larger study to confirm or refute the hypothesis that genetic variation in *CCR5* is associated with the prevalence of antibody to varicella-zoster virus.

Study participants were enrolled in the Retrovirus Epidemiology Donor Study (REDS) sponsored by the US National Heart Lung and Blood Institute. REDS is a multicentre study of the prevalence of infectious agents in blood donors. Testing was done on specimens that had been dissociated from any information that could identify an individual. We determined the *CCR5-Δ32* genotype of 1109 white blood donors who were born before 1957. We tested antibodies to varicella-zoster virus in all 210 *CCR5-Δ32* carriers (14 homozygotes and 196 heterozygotes), and in 210 age-matched *CCR5* wild-type individuals. Serum samples from all study participants were tested for IgG, IgM, and IgA to varicella-zoster virus with ELISA methods developed at the US Centers for Disease Control and Prevention. All ELISA assays were done with whole-cell antigen preparations of human fibroblasts infected with varicella-zoster virus. IgM and IgA assays were done with a capture ELISA format to eliminate interference by IgG. A specimen was regarded as positive if its optical density exceeded 0·166.

The mean age was 63·4 years (SD 9·0, range 50–79) for the *CCR5-Δ32* homozygotes, 58·1 years (9·0, 48–87) for the *CCR5-Δ32* heterozygotes, and 59·3 years (2·0, 57–63) for the wild-type controls. Six (43%) of 14 *CCR5-Δ32* homozygotes, 116 (59%) of 196 *CCR5-Δ32* heterozygotes, and 132 (63%) of 210 wild-type individuals were men. None of the participants tested were positive for IgM or IgA to varicella-zoster virus, and all except two individuals—one *CCR5-Δ32* homozygote and one *CCR5* wild-type individual—were positive for IgG to varicella-zoster virus (table). The serum prevalence of IgG to varicella-zoster virus was therefore 99·5% (95% CI 97·4–100·0) for both *CCR5-Δ32* carriers (92·9% for homozygotes and 100·0% for heterozygotes) and for *CCR5* wild-type individuals. The difference in seroprevalence between *CCR5-Δ32* homozygotes or heterozygotes and wild-type individuals was not significant (p=0·12 and p=0·52, respectively, Fisher's exact test).

We also examined optical density readings among the participants to find out whether antibody reactivity differed by *CCR5* genotype. Although optical density readings from an ELISA do not represent antibody titres linearly within all ranges, a high optical density does represent a high antibody titre. We classified individuals who were seropositive for varicella-zoster virus into three categories: low optical density (0·167–0·400), moderate optical density (0·401–0·700), and high optical density (>0·701). None of the *CCR5-Δ32* homozygotes, and only two each of *CCR5-Δ32* heterozygotes and wild-type individuals had low optical densities (table). We also compared the distribution of the ELISA optical density for IgG to varicella-zoster virus among *CCR5-Δ32* carriers and controls. The median optical density was 1·624 for *CCR5-Δ32* carriers and 1·520 for wild-type individuals (p=0·07, Wilcoxon's rank-sum test).

In this study of US blood donors, almost all individuals had antibody to varicella-zoster virus and there was no evidence of a lower varicella-zoster virus seroprevalence or lower varicella-zoster virus IgG reactivity among people who carried the *CCR5-Δ32* allele. Our results contrast with those of Wiencke and colleagues, who found an overall seroprevalence of 90% in 157 blood donors (mean age 53 years) and a significant difference in genotype-specific seroprevalence (95·3% in wild-type individuals and 69·0% in *CCR5-Δ32* carriers).[4] Our findings of a high varicella-zoster virus seroprevalence irrespective of *CCR5* genotype are more consistent with those of a study by Nguyen and colleagues, in which 12 of 13 HIV-1-uninfected *CCR5-Δ32* homozygotes were seropositive for varicella-zoster virus.[5] Our findings are also consistent with previous estimates of varicella-zoster virus seroprevalence in the USA, which reaches almost 100% by middle age. For example, the varicella-zoster virus seroprevalence was about 99·5% in people older than 40 years who were enrolled in the US Third National Health and Nutrition Examination Survey, which was carried out from 1988 to 1994 (unpublished data, US Centers for Disease Control and Prevention). Because the *CCR5-Δ32* allele is found in about 20% of white people,[1] population seroprevalence estimates approaching 100% are inconsistent with the hypothesis that this allele is linked to a marked reduction in seroprevalence of varicella-zoster virus. In all, the data do not seem to support a role for the *CCR5-Δ32* allele in varicella-zoster virus infection.

Contributors

M Zhang designed the study, analysed and interpreted data, and wrote the paper; S Schmid determined anti-VZV antibody assay results, contributed to interpretation of data, and reviewed the paper; M Carrington determined *CCR5*-Δ32 genotype and reviewed the paper. T R O'Brien designed the study and supervised all of its aspects.

Conflict of interest statement

None declared.

Acknowledgments

We thank James Goedert for helpful discussions and review of the draft manuscript; Diane Wang for *CCR5* genotyping; Denise Brown and Martha Thieme for ELISA testing for varicella-zoster virus antibodies; and the REDS study investigators for providing specimens. This publication has been funded in part with federal funds from the National Cancer Institute, NIH, under contract number NO1-CO-56000.

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Association between genetic variation in the gene for insulin-like growth factor-I and low birthweight

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Low birthweight is associated with later risk of type 2 diabetes and related disorders. We aimed to show that a polymorphism in the gene for insulin-like growth factor-I, which has proved to raise risk of type 2 diabetes and myocardial infarction, is associated with low birthweight. We recorded birthweight and obtained DNA for 463 adults. Individuals who did not have the wild-type allele of the polymorphism had a 215 g lower birthweight than those homozygous for this allele (95% CI –411 to –10). Our data lend support to the hypothesis that genetic variation affecting fetal growth could account for the association between low birthweight and susceptibility to diabetes and cardiovascular disease in later life.

Lancet 2002; **359:** 1036–37

The finding that low birthweight is a strong predictor of diabetes and cardiovascular disease in later life has led to continuing debate about the role of nature and nurture. Barker[1] has argued that poor nutrition in utero—leading to low birthweight—predisposes an infant to diabetes and cardiovascular disease. However, Hattersley and Tooke[2] have postulated that fetal genetic factors could underlie this relation.

Insulin-like growth factor-I (IGF-I) and insulin are essential fetal growth factors.[3] IGF-I is also important for growth and development of insulin-producing β cells in the pancreas. Furthermore, a shortage of IGF-I has been implicated in the pathogenesis of type 2 diabetes and cardiovascular disease in later life.[4] We have shown that absence of the wild-type (192 bp) allele of a polymorphism in the promoter region of the *IGF-I* gene results in low circulating IGF-I concentrations, reduced height in adulthood, diminished insulin-secreting capacity, and a high risk of type 2 diabetes and myocardial infarction.[4] We postulated that if this polymorphism affects fetal IGF-I and insulin secretion, it could also have a role in regulation of birthweight, and could thus explain part of the relation between low birthweight and type 2 diabetes mellitus and cardiovascular disease later in life. We investigated the relation between birthweight and the IGF-I-promoter polymorphism genotype.

	***IGF-I* promoter polymorphism genotype**		
	Homozygous 192 bp allele (n=195)	Heterozygous 192 bp allele (n=204)	No 192 bp allele (n=64)
Postnatal gain in weight			
Yes	19 (10%)	29 (14%)	17 (27%)
No	76 (90%)	175 (86%)	47 (73%)

Data are number of participants (%). All individuals were categorised according to quartiles of birthweight and adult weight distribution.

Table 2: **Number of participants who showed a postnatal gain in weight during life, stratified by genotype**

Our study is based on a sub-study of the Rotterdam Study,[4] which is focusing on the pathogenesis of diabetes in 1110 people. Data for birthweight and DNA were obtained for 463 of these participants, including 93 with type 2 diabetes. Clinical data were obtained without knowledge of genotype. Information about health status, birthweight, and family history of diabetes mellitus was obtained with a standard questionnaire. Height and weight were measured and body-mass index was calculated. We obtained written informed consent and the local ethics committee approved the study. Genotype effect size was evaluated with the *t* test or the Mann-Whitney *U* test. Interaction between genotypes was measured by a two-factor ANOVA analysis.

Table 1 shows the effect of *IGF-I* genotype on birthweight (p=0·05). Absence of the 192 bp allele was associated with a 215 g reduction in birthweight compared with individuals homozygous for this allele (95% CI –411 to –10). People who were heterozygous and homozygous for the wild-type allele did not differ significantly.

The difference in birthweight between genotypes was most striking for individuals with a history of diabetes in the mother (p=0·001). Mean birthweight was higher for individuals with a history of maternal diabetes (3431 g [SD 806]) than for those with no maternal history of diabetes (3234 g [696]; p=0·03). In the group of individuals with a history of diabetes in the mother, absence of the 192 bp allele was associated with a 600 g lower birthweight than those who were homozygous for this allele (95% CI –1184 to –15; p=0·03).

No association was recorded between genotype and weight or obesity (body-mass index >27 kg/m^2) in adulthood. However, IGF-I genotype was strongly associated with postnatal weight gain, defined as a shift within the weight distribution to a higher quartile (table 2). Of individuals homozygous for the 192 bp allele, just under a tenth showed a shift towards the mean weight of the general population during life. In individuals without the 192 bp allele, this weight gain was seen for over a quarter of individuals (p=0·001).

Absence of the wild-type allele of a polymorphism in the promoter of *IGF-I* has proved to result in low IGF-I expression and high risk of type 2 diabetes mellitus and myocardial infarction in old age.[5] We have shown that this allele is also associated with fetal growth. This finding suggests that IGF-I is important for fetal growth. However, it does not exclude the fact that other growth factors (such as IGF-II) could also have a role in fetal growth, or that IGF-I could be associated with fetal growth by regulation of other hormones such as IGF-II or insulin.[3] There is evidence that IGF-II, which shows high affinity for the IGF-I receptor, could be a factor in fetal insulin secretion, and could thus be implicated in fetal growth.[5]

	All			**Participants with a history of maternal diabetes**		
	n	Mean difference (95% CI)	p*	n	Mean difference (95% CI)	p†
***IGF-I* genotype**						
Homozygous 192 bp allele	195	3299 (3196–3402)	..	37	3442 (3173–3657)	..
Heterozygous 192 bp allele	204	3296 (3196–3396)	0·74	42	3488 (3229–3714)	0·83
No 192 bp allele	64	3084 (2934–3234)	0·04	10	2842 (2354–3330)	0·03

Genotypes based on presence of wild-type (192 bp) allele. *Based on *t* test; †Based on Mann-Whitney *U* test.

Table 1: **Association between a polymorphism in IGF-I and birthweight**